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THE IMPACT OF HABITAT CONDITIONS ON THE PERFORMANCE OF GENERATIVE RAMET CLUSTERS OF HIGH MEDICINAL VALUE, RARE SPECIES BETONICA OFFICINALIS L.

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Abstract

Observations were carried out in the years 2014-2015 in moor grass meadows, old fields, willow thickets and macroforbs. In the successive study sites, the height of the standing vegetation and soil moisture gradually increased, whilst the light availability decreased. Stable in consecutive seasons, the total number of ramets per generative ramet cluster achieved the lowest values in willow thickets due to mechanical suppression of vegetative growth by the robust underground organs of neighbouring plants. Constant during the study period, the share of generative stems decreased in successive study sites, while the percentage of leaf rosettes showed an inverted tendency. The increase of height of generative stems and number of flowers per inflorescence in consecutive Patches, as well as the augmentation of length of flowers in time and space, might trigger an improvement of generative reproduction in a crowded environment. The increase of the number of nodes and dimensions of cauline leaf blades in successive Patches and the augmentation of the number and dimensions of rosette leaves in the time and space might contribute to greater efficacy of light capture in growing shading. Summarizing the plasticity of traits might assure the spread of generative ramet clusters in open habitats, as well as their persistence in crowded sites.

Keywords: *Cauline leaves; Flowers; Generative stem; Rosette; Spatial and temporal variability*

Introduction

The majority of herbaceous taxa such as mosses, ferns and angiosperms, belong to clonal plants, which might propagate in vegetative and generative ways [1]. The asexual propagation assures longtime persistence in a colonised site, while sexual reproduction contributes to augmentation of genetic variability of populations and dispersal into new areas [2, 3]. Since the groundbreaking publications of Eriksson [4, 5], bringing evidence that sexual reproduction in populations of clonal plants may be frequent, the amount of research of the effectiveness of generative propagation increased remarkably. The large body of data indicates that the size of seeds, their germination capacity, as well as the recruitment and survivability of seedlings might vary in relation to different habitat conditions i.e. the character of adjacent plants [6-12], successional stage [13], climatic conditions [14-17], altitude above soil level [18], light intensity [19], soil humidity [20], as well as salinity level [21].

Simultaneously, it should be pointed out that a much smaller number of authors [22-28] carried out the observations of traits of generative individuals such as number of flowering stalks, size of inflorescences, flower production etc. The obtained results allow for describing

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the inter-population variability of the studied features, characterising the adjustments of generative individuals to a changing environment, as well as estimating their state and predicting the chances for maintenance in a colonised site. From this point of view, the studies carried out in populations of threatened taxa are very valuable, but the greatest meaning brings observations conducted in populations of rare, useful plants. So far, such detailed, multi-faceted investigations were carried out in populations of *Arnica montana* [29] and *Valeriana jatamansi* [30].

Although there is increasing interest in the aforementioned topic, the present state of knowledge is still insufficient and further investigations are strongly needed. One of the increasingly rare and widely useful plants in medicine is *Betonica officinalis* L., which preliminary observations showed the remarkable differences in structure of subpopulations in course of secondary succession [31]. These findings have inspired to the continuation of studies, which main goal became an assessment of the effect of habitat conditions on the performance of generative ramet clusters. The specific aims were concentrated on: evaluation of the temporal and spatial variability of: (i) the number and share of leaf rosettes and generative stems in ramet clusters, (ii) the traits connected with generative propagation (height of main axis of generative stem, number of axillary branches topped with inflorescences, number of flowers per inflorescence, length of flowers), and (iii) number of cauline leaf nodes and leaf rosettes, as well as the dimensions of leaf blades.

Study species

Wood betony *Betonica officinalis* syn. *Stachys officinalis* (L.) Trevis is a perennial, clonal herb preferring damp, slightly acidic and poor soils. It occurs in dry grassland, lightly grazed pastures, meadows and open woods in Europe and Western Asia [32]. *Betonica officinalis* is a medicinal plant which has been used for centuries for the treatment of disorders of the respiratory, gastrointestinal tract, cerebral afflictions, high blood pressure, skin ulcers and infected wounds, as well as gynecological problems [33-35]. As a result of the considerable exploitation for medicinal purposes, this increasingly rare taxon in the whole of Europe is included in the European Red List of Medicinal Plants [36] and in the several national Red Lists and Books [37-41].

Betonica officinalis is classified among rhizomatous species creating aboveground units such as rosettes of leaves and upright and flowering stems with oppositely paired oblong, stalkless, cauline leaves. The often branched, hairy generative stems growing up to 90 cm are topped by interrupted spikes formed by several rings or whorls of bright, purple-red or occasionally white flowers. The monopetalous bi-labiate corolla attains 12–16 mm [42]. The flowers are pollinated by bumblebees (mostly *Bombus pascuorum*). *Betonica officinalis* spreads notably by means of seeds and by slow and steady vegetative growth leading to the division of the senile parts of rhizomes into independent parts [42, 43].

Considering the opportunity for vegetative growth, the individual and the ramet cluster were adopted as the basic demographic units in the presented investigations. An individual (genet) is a plant emerging from a single zygote. The terms ‘individual’ and ‘genet’ are used in association with the juvenile stage, since only at this stage can it be established with certainty that it has developed from a zygote. The term ‘ramet cluster’, understood as an integral group of the aboveground units (leaf rosettes or/and generative stems), is applied to plants representing later developmental stages (vegetative and generative).

Material and methods

Study area

Studies were carried out in the south-western part of Krakow (southern Poland) between Pychowice and Tyniec (Fig. 1) on a low flood terrace of the Vistula. The peaty or clay- and silt-

laden soils common in the Vistula valley are covered by *Molinion caeruleae* Patches [44]. The abandonment of traditional land use over at least the past dozen years contributed to the encroachment of *Phragmites* swamps and willow brushwood, leading to the fragmentation of meadows [45, 46].

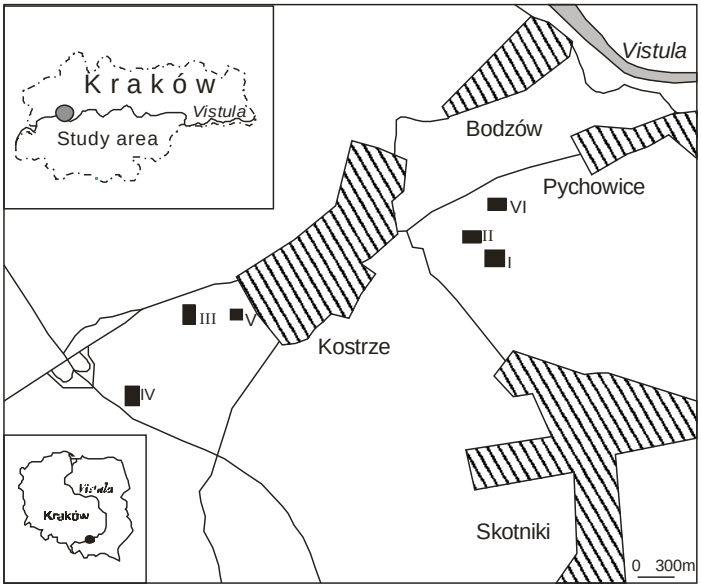

Fig. 1. The localities of subpopulations of *Betonica officinalis* L. in moor grass meadow (I), overgrowing moor grass meadow (II), oldfields (III, IV), willow thickets (V), macroforbs (VI).

Numerous plant communities have formed in the vicinity of the meadows, from the deciduous forests covering the slopes of the Vistula valley, through the xerothermic calcareous grasslands occurring on the limestone and chalk hill slopes, to the ruderal communities appearing near buildings and alongside the edges of roads. The research area involved various plant communities from abandoned wet meadows (Patches I and II), via old fields (Patches III and IV) and thickets (Patch V), to macroforbs (Patch VI) characterised by similar density and age structure of *Betonica officinalis* subpopulations ($\chi^2 = 1.166$, $df = 5$) (Table 1).

Characteristics of habitat conditions

In all Patches, in July 2014, one permanent plot measuring 15 x 15m was established and fenced in. Within each plot, 30 measuring points were chosen, labelled by plastic pegs. Five of them were established systematically in the middle and in the corners of permanent plots, while the remaining was selected randomly. The marked points were centres of setting the square aluminum frame measuring 30 x 30cm, which was used for visual evaluation of the plant cover and average height of the vascular plants. Each time the percent of ground surface covered by the vascular plants, moss and lichens within the frame was estimated. The average height of the vascular plants was calculated on the basis of the measurement of total dimensions (from soil level to the top) of the lowest and the highest stem (excluding *Betonica officinalis*) growing within the frame. Altogether, 60 measurements using a folding tape measure were taken within each plot.

In the aforementioned points the measurements of the light intensity at soil level and soil moisture were carried out. The light intensity was examined with a Voltcraft MS-1300 digital light meter (accuracy ± 5% + 10 digits; range 0.01 – 50,000lx). The humidity at ground level was evaluated on the basis of 30 measurements taken randomly using a hand-held BIOWIN

071505 soil moisture sensor (measuring range 1-10). In both study years the measurements were performed on July 27 between 10.00 and 12.00 a.m. In the successive study sites, the height of the standing vegetation and soil moisture gradually increased, whilst the light availability decreased. A detailed description of the habitat conditions within the permanent plots established in the particular study Patches is given in Table 2.

Table. 1. The characteristics of studied Patches.

Patch name		I	II	III	IV	V	VI
Patch area (m^2)		900	1400	900	1300	800	1300
Plant association		Moor-grass meadow	Overgrowing moor-grass meadow	Oldfield	Oldfield	Willow thickets	Macroforbs
Number of species per Patch		63	52	45	28	21	24
Dominants (species, with cover >20%)		*Centaurea jacea, Succisa pratensis, Iris sibirica,*	*Sanguisorba officinalis, Iris sibirica, Molinia caerulea,*	*Solidago canadensis, Heracleum sphondylium, Arrhentherum elatius*	*Calamagrostis epigeios, Sanguisorba officinalis,*	*Salix repens ssp. rosmarinifolia Salix caprea,*	*Filipendula ulmaria, Lysimachia vulgaris,*
Subdominants (species, with cover 5-20%)		*Gladiolus imbricatus, Potentilla erecta*	*Lysimachia vulgaris, Serratula tinctoria*	*Achillea millefolium, Erigeron annua*	*Solidago canadensis*	*Betula pendula, Salix cinerea*	*Phragmites australis, Sanguisorba officinalis*
Abundance of ramet clusters of *Betonica officinalis* L. in Patches		115	217	125	150	53	124
Mean number of ramet clusters of *Betonica officinalis* L. per m^2		0.12	0.15	0.13	0.11	0.06	0.09
Age structure of *Betonica officinalis* L subpopulations	I	0	0	0	0	0	0
	J	0	0	0	0	0	0
	V	28.7	24.7	23.2	25.4	22.7	21.8
	G	71.3	75.3	76.8	74.6	77.3	78.2

Abbreviations mean particular age stages: I - initial, J - juvenile, V - vegetative, G - generative

Table 2. The characteristics of habitat conditions in permanent plots established in particular Patches.

Patch name	**Year**	**I**	**II**	**III**	**IV**	**V**	**VI**
The mean plant cover (%)	2014**	99.5 (±1.8) [a]	99.4 (±2.4) [a]	94.0 (±12.0) [a]	99.3 (±2.2) [a]	99.1 (±2.6) [a]	98.8 (±4.0) [a]
	2015**	9.6 (±1.4) [a]	99.4 (±2.8) [a]	94.3 (±11.7) [a]	99.4 (±1.9) [a]	99.2 (±2.6) [a]	99.8 (±2.4) [a]
The mean vascular plant height (cm)	2014***	35.0 (±13.8) [a]	51.9 (±22.6) [a]	66.8 (±14.3) [a]	97.2 (±29.6) [a]	89.2 (±20.9) [a]	115.6 (±37.2) [a]
	2015***	37.3 (±12.9) [a]	55.3 (±19.8) [a]	71.0 (±12.0) [b]	109.6 (±20.8) [b]	99.1 (±27.1) [b]	128.5 (±36.8) [b]
The mean light intensity at the soil level (lx)	2014***	35 500 (±8 200) [a]	32 900 (±4 700) [a]	30 600 (±8 100) [a]	32 500 (±5 900) [a]	27 800 (±5 800) [a]	26 700 (±6 200) [a]
	2015***	30 6300 (±10 170) [b]	30 230 (±6 020) [a]	28 300 (±8 240) [a]	27 760 (±4 020) [b]	22 000 (±9 120) [b]	21 930 (±3 400) [b]
The mean soil humidity (range 1-10)	2014***	2.5 (±0.5) [a]	3.4 (±0.7) [a]	3.3 (±0.6) [a]	4.3 (±0.6) [a]	5.3 (±0.5) [a]	5.0 (±0.7) [a]
	2015***	2.3 (±0.5) [a]	3.0 (±0.6) [a]	3.1 (±0.9) [a]	4.1 (±1.7) [a]	5.0 (±0.3) [a]	4.8 (±0.7) [a]

Asterisks mean the statistical significance of differences (the one-way ANOVA) among Patches in particular years: **- P <0.01; ***- P <0.001. The same letters mean lack of remarkable statistical differences (T - Student test) among years.

Characteristics of ramet clusters

On July 28, 2014, in each of the aforementioned permanent plots, all the generative ramet clusters were labelled with plastic pegs for further observations. Each year all of the below mentioned traits were observed:

a) the height of the generative stems (from the soil surface to the top of the main axis), the number of axillary branches topped by inflorescences and the number of flowers per each inflorescence, as well as the maximal length (from a pedicel to the upper lip of the corolla) of 10 randomly chosen flowers taken from each inflorescence;

b) the number of cauline leaf nodes, as well as the length and width of the blade of one selected uppermost and one lowermost cauline leaf in each generative stem;

c) the number of leaves per rosette and the maximal length and width of each leaf blade.

The measurements of the height of the generative stems were carried out using a folding tape measure, while the dimensions of the leaf blades and flowers were measured using a Lux Tools Comfort digital calliper (accuracy ±0.01; measuring range 0.1–150mm).

Statistical analysis

The chi-square statistic was applied to test whether significant differences existed among the sites in terms of the share of:

- leaf rosettes and flowering stems in the generative ramet clusters,
- generative stems with different height,
- flowers with different length.

Normal distribution of the untransformed data of each studied trait in an individual sample (from a particular Patch and year) was tested using the Kolmogorov-Smirnov test, while variance homogeneity was tested using the Levene test at the significance level of $P<0.05$. As the values of the individual characteristics in some groups were not consistent with normal distribution and the variance was not homogeneous, the U Mann-Whitney test was used to check if there is temporal variability, whereas the Kruskal-Wallis test was applied to check whether there is spatial variability in terms of: the total number of aboveground units per ramet cluster; the number of inflorescences per generative stem and the number of flowers per inflorescence; the number of cauline leaf nodes per generative stem; the length/width of the uppermost/lowermost leaves; and the number of leaves per rosette.

The one-way analysis of variance (ANOVA) was used to determine if there are significant differences among the sites in terms of: the length of rosette leaves and the width of the rosette leave. The Student test was used to determine if there are significant differences among the years in terms of: the length of rosette leaves, the width of the rosette leaves, and the length of the flowers. Statistical analyses were performed using *STATISTICA 10* software [47].

Results

Number and share of leaf rosettes and generative stems in ramet clusters

The total number of aboveground ramets in the generative ramet clusters did not show temporal variability. In Patches I-IV and VI it was quite similar and meanly amounted 2.30, 2.36, 2.20, 2.23 and 2.42 in the year 2014, as well as 2.10, 2.31, 2.27, 2.38 and 2.17 in the year 2015. In Patch V the total number of aboveground ramets was substantially lower and attained 1.71 and 2.09 in consecutive seasons. The contribution of generative stems and leaf rosettes per ramet cluster were rather constant in consecutive years. The share of generative stems diminished, while the contribution of leaf rosettes rose gradually from Patch I, *via* Patches I, III, IV and VI, to Patch V (Fig. 2).

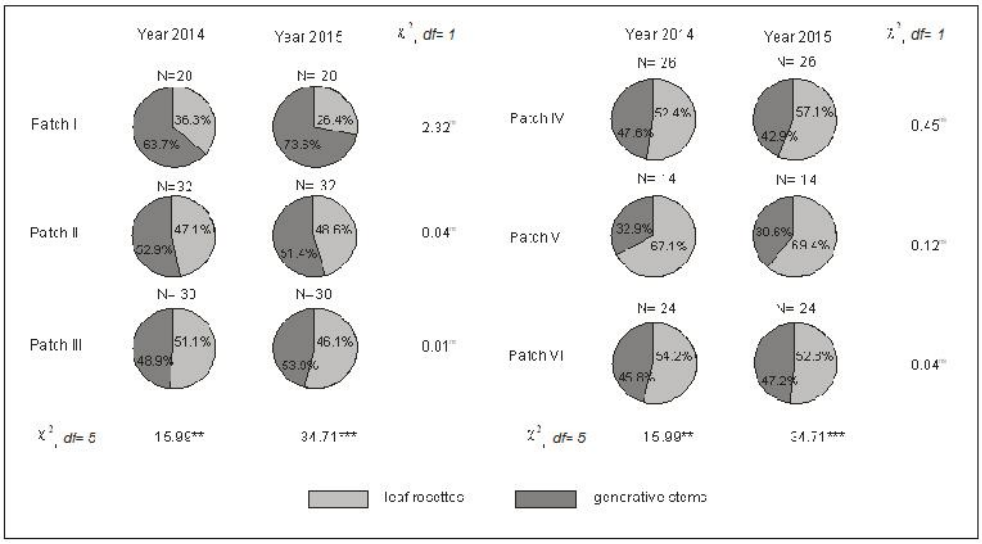

Fig. 2. The contribution of leaf rosettes and generative stems in ramet clusters growing in permanent plots within particular Patches in the years 2014 and 2015.

Traits connected with generative propagation

The height of the generative stems achieved from 30 to 109cm. It rather did not differ significantly among the years, although it differed in the study sites. Significantly lower stems were noticed in Patches I, II and IV than in sites III, V and VI (Figure 3).

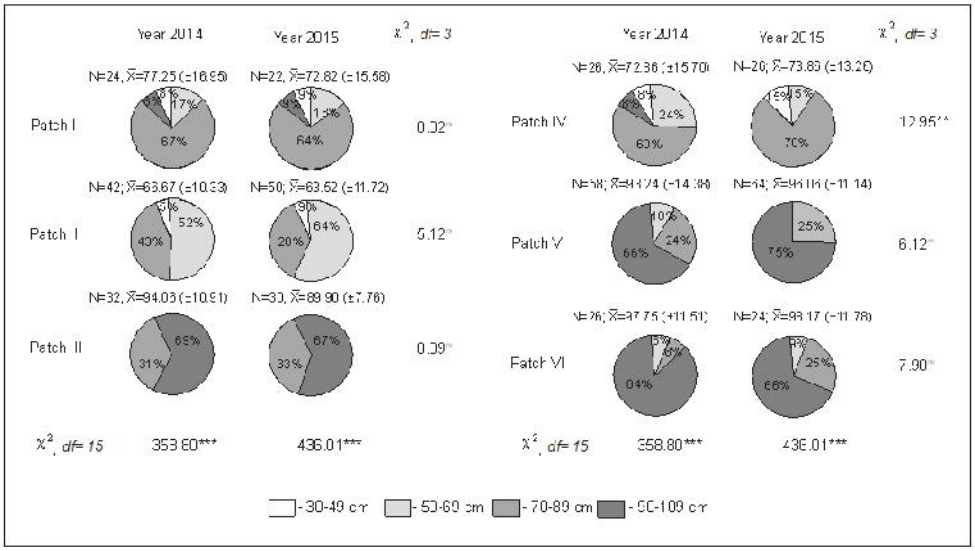

Fig. 3. The contribution of generative stems with different height in studied subpopulations of *Betonica officinalis L.* in the years 2014 and 2015.

The number of branches per generative stem did not show the temporal nor the spatial variability, whilst the number of flowers per inflorescence did not differ among seasons but it generally increased in successive Patches (Table 3). The lowest number of flowers, amounting to 60.00 and 57.73 on average, was observed in Patch I, whereas the greatest number, reaching an average of 105.33 and 122.75, was found in Patch VI. The length of flowers increased in

successive Patches, excluding Patch V where flowers achieved the lowest dimensions. In majority of subpopulations the flower dimensions were rather constant in both study years (Figure 4).

Table 3. The mean numbers of branches of generative stem (±SD) and the mean number of flowers per inflorescence (±SD) in studied subpopulations of *Betonica officinalis* L. in the years 2014 and 2015.

	Number of branches		The level of statistical significance among years (Mann-Whitney test)	Number of flowers		The level of statistical significance among years (Mann-Whitney test)
	Year 2014	Year 2015		Year 2014	Year 2015	
Patch I	1.00 (±0.00) N = 24	1.00 (±0.00) N = 22	Z = 0.00 ns	60.00 (±17.75) N = 24	57.73 (±19.15) N = 22	Z = 0.91 ns
Patch II	1.00 (±0.00) N = 42	1.04 (±0.20) N = 50	Z = -0.32 ns	61.67 (±16.64) N = 42	56.00 (±20.81) N = 52	Z = 0.66 ns
Patch III	1.17 (±0.42) N = 32	1.20 (±0.84) N = 30	Z = -0.17 ns	101.71 (±41.64) N = 34	86.58 (±43.53) N = 38	Z = 1.75 ns
Patch IV	1.00 (±0.00) N = 26	1.00 (±0.00) N = 26	Z = 0.00 ns	104.00 (±35.63) N = 26	120.57 (±50.30) N = 26	Z = -1.05 ns
Patch V	1.07 (±0.26) N = 58	1.00 (±0.00) N = 64	Z = 0.65 ns	83.77 (±12.89) N = 62	95.75 (±14.82) N = 64	Z = -1.21 ns
Patch VI	1.00 (±0.00) N = 26	1.00 (±0.00) N = 24	Z = 0.00 ns	105.33 (±34.36) N = 26	122.75 (±46.70) N = 24	Z = -1.69 ns
The level of statistical significance of differences among Patches (Kruskal-Wallis test)	H = 6.55 ns	H = 8.94 ns		H = 66.89 ***	H = 62.31 ***	

Abbreviations: N-number of measurements,
The statistical significance level: *-P<0.05, **-P<0.01, ***-P<0.001, ns-not significant

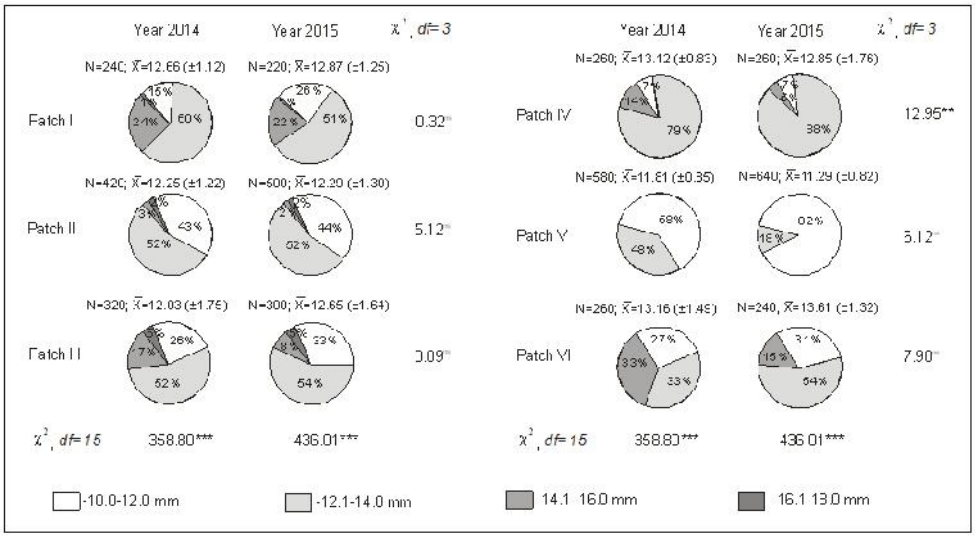

Fig. 4. The contribution of flowers with different length in studied subpopulations of *Betonica officinalis* L. in the years 2014 and 2015.

Cauline and rosette leaves

The number of nodes of the cauline leaves in consecutive years was rather constant, and it was the lowest in Patch I (Figure 5). The length and width of the uppermost (Table 4) as well as the lowermost cauline leaves (Table 5) in consecutive years were rather stable, but they showed a spatial variability. The smallest leaves were found in Patch I, whereas the largest ones were found in Patch V.

Table 4. The mean length and width of uppermost cauline leaf [mm] (±SD) in studied subpopulations of *Betonica officinalis* L. in the years 2014 and 2015.

	Length		The level of statistical significance among years (U-Mann-Whitney test)	Width		The level of statistical significance among years (Mann-Whitney test)
	Year 2014	Year 2015		Year 2014	Year 2015	
Patch I	34.39 (±10.13) N = 24	33.76 (±11.66) N = 22	Z = 0.47 ns	10.30 (±6.36) N = 24	12.41 (±6.21) N = 22	Z = -1.13 ns
Patch II	38.59 (±13.58) N = 42	36.91 (±13.00) N = 50	Z = 0.65 ns	15.91 (±7.86) N = 42	14.80 (±6.55) N = 50	Z = 0.63 ns
Patch III	58.39 (±10.02) N = 32	56.52 (±9.02) N = 30	Z = 0.78 ns	29.23 (±6.59) N = 32	25.57 (±6.76) N = 30	Z = 0.13 ns
Patch IV	53.89 (±14.16) N = 26	54.75 (±15.64) N = 26	Z = 0.37 ns	25.27 (±4.72) N = 26	22.13 (±5.08) N = 26	Z = 1.05 ns
Patch V	63.50 (±7.81) N = 58	66.28 (±9.53) N = 64	Z = 0.43 ns	34.87 (±6.01) N = 58	31.98 (±6.05) N = 64	Z = 0.86 ns
Patch VI	54.40 (±12.63) N = 26	51.90 (±14.66) N = 24	Z = 1.73 ns	31.27 (±10.65) N = 26	26.27 (±9.35) N = 24	Z = 2.43*
The level of statistical significance of differences among Patches (Kruskal-Wallis test)	H = 105.35 ***	H = 117.11 ***		H = 115.13 ***	H = 124.10 ***	

Abbreviations as in Table 3.

The number of leaves per rosette was rather similar in both study seasons, but it differed remarkably among the Patches. It augmented in the consecutive sites and amounted, on average, from 3.36 to 8.09 in the year 2014, and from 3.88 to 8.46 in the year 2015 (Figure 6). The length and width of leaves increased in consecutive seasons and in successive Patches (Table 6). The smallest length of leaves was noted in Patch I, where it amounted to 73.03 mm and 74.27 mm. The greatest length was recorded in Patch VI, where it measured 99.64 mm and 102.58 mm, respectively. The smallest width of leaves was found in Patch I, where it measured 42.17 mm and 42.86 mm, whilst the greatest width was observed in Patch VI, where it reached 66.37 mm and 67.02 mm, respectively.

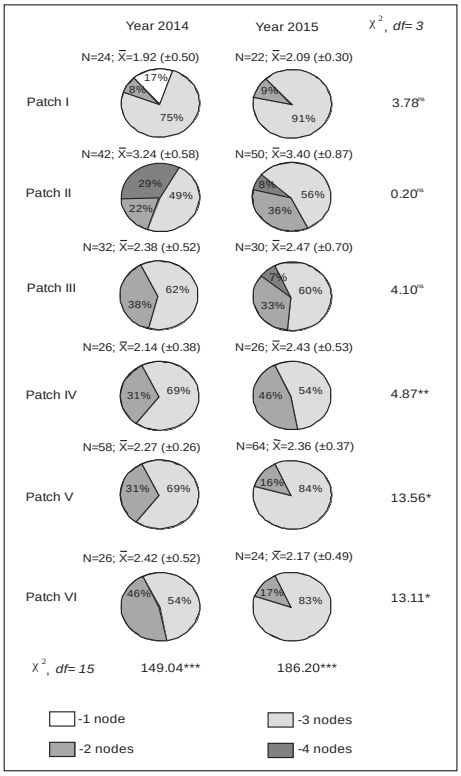

Fig. 5. The contribution of generative stems with different number of nodes of cauline leaves in studied subpopulations of *Betonica officinalis* L. in the years 2014 and 2015.

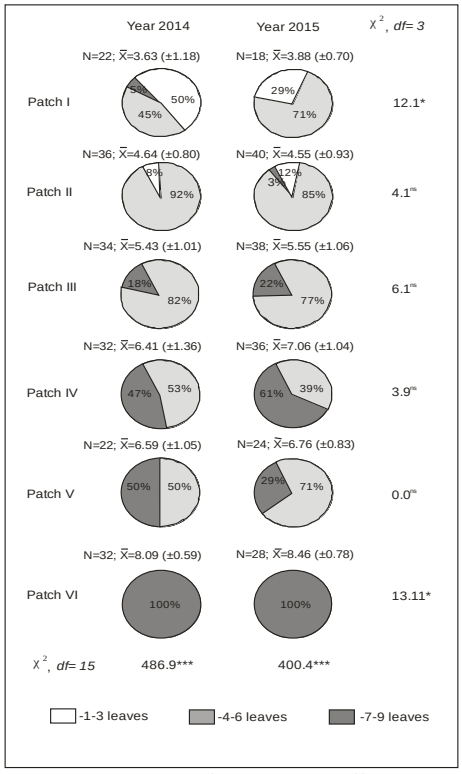

Fig. 6. The contribution of rosettes with different number of leaves in studied subpopulations of *Betonica officinalis* L. in the years 2014 and 2015.

Table 5. The mean length and width of lowermost cauline leaf [mm] (±SD) in studied subpopulations of *Betonica officinalis* L. in the years 2014 and 2015.

	Length		The level of statistical significance among years(Mann-Whitney test)	Width		The level of statistical significance among years(Mann-Whitney test)
	Year 2014	Year 2015		Year 2014	Year 2015	
Patch I	56.27 (±12.38) N = 24	58.48 (±12.45) N = 22	Z = -0.12 ns	25.67 (±8.07) N = 24	29.13 (±7.64) N = 22	Z = -0.12 ns
Patch II	71.02 (±18.22) N = 42	64.90 (±19.53) N = 50	Z = 0.95 ns	26.43 (±7.87) N = 42	29.65 (±9.02) N = 50	Z = -1.56 ns
Patch III	81.55 (±15.30) N = 32	79.63 (±14.01) N = 30	Z = 0.27 ns	43.51 (±5.99) N = 32	41.79 (±4.05) N = 30	Z = 1.20 ns
Patch IV	76.86 (±11.61) N = 26	87.57 (±13.78) N = 26	Z = -0.97 ns	47.62 (±6.84) N = 26	45.05 (±9.79) N = 26	Z = 0.50 ns
Patch V	87.75 (±9.34) N = 58	90.65 (±10.94) N = 64	Z = -0.60 ns	47.12 (±5.46) N = 58	46.47 (±4.84) N = 64	Z = 0.51 ns
Patch VI	74.65 (±14.33) N = 26	72.15 (±13.06) N = 24	Z = 0.88 ns	45.31 (±9.17) N = 26	40.31 (±8.61) N = 24	Z = 2.20 *
The level of statistical significance of differences among Patches (Kruskal-Wallis test)	H=69.20 ***	H=77.09 ***		H=112.31 ***	H=100.44 ***	

Abbreviations as in Table 3.

Table 6. The mean length and width of rosette leaves [cm] (±SD) in studied subpopulations of *Betonica officinalis* L. in the years 2014 and 2015.

	Length		The level of statistical significance among years (Student test)	Width		The level of statistical significance among years (Student test)
	Year 2014	Year 2015		Year 2014	Year 2015	
Patch I	73.07 (±11.14) N = 81	74.27 (±6.47) N = 66	t = -0.77 ns	48.17 (±12.62) N = 81	42.86 (±6.98) N = 66	t = 3.05**
Patch II	72.75 (±4.73) N = 167	76.57 (±10.35) N = 182	t = -4.36**	42.90 (±4.73) N = 167	46.01 (±6.42) N = 182	t = -5.04***
Patch III	84.60 (±12.05) N = 201	87.47 (±5.18) N = 211	t = -3.16**	52.36 (±6.18) N = 201	56.91 (±4.07) N = 211	t = -8.86***
Patch IV	85.49 (±9.67) N = 205	92.11 (±10.44) N = 254	t = -7.38***	60.72 (±5.46) N = 205	61.89 (±7.30) N = 254	t = 3.59 ns
Patch V	94.12 (±10.20) N = 145	95.81 (±8.73) N = 163	t = -1.26 ns	64.23 (±5.87) N = 145	67.43 (±4.09) N = 163	t = -1.89 ns
Patch VI	99.64 (±9.43) N = 259	102.58 (±12.18) N = 237	t = -3.03*	66.37 (±6.01) N = 259	67.02 (±6.19) N = 237	t = -1.18 ns
The level of statistical significance among years (one-way ANOVA)	F = 184.9 ***	F = 214.8 ***		F = 361.9 ***	F = 438.4 ***	

Abbreviations as in Table 3

Discussion

Number and share of leaf rosettes and generative stems in ramet clusters

The observed, much lower number of aboveground units of *Betonica officinalis* in willow thickets than the other sites might be linked with inhibition of clonal growth by the creeping, woody stems of *Salix repens* ssp. *rosmarinifolia*, as well as the robust undergrown organs of other shrubs and trees. The suppression of vegetative growth – an effect of exposure to mechanical stress provided by superior competitors - was observed in numerous stoloniferous [48, 49] and rhizomatous species [50]. *B.B. Casper and R.B. Jackson* [51] argued that belowground competitive ability, particularly correlated with the growth, density and surface of roots, might be also linked with the occurrence of enzymes involved in nutrient uptake. Moreover, the aforementioned authors claimed that belowground competition can be stronger and involve many more neighbours than aboveground.

The greatest contribution of generative stems of *Betonica officinalis* noticed in the present study was in meadows dominated by low-statured taxa with sparsely arranged shoots, which might be consequence of substantial investment in generative propagation in the vicinity of minor competitors. A much lower share of flowering stalks in the overgrowing meadows, old fields and macroforbs seems to be a result of diminished allocation to sexual propagation in crowded habitats dominated by plants reaching great dimensions. The lowest contribution of generative stems of *Betonica officinalis* in willow thickets might be a consequence of the slight investment in generative propagation in the vicinity of individual *Salix repens* ssp. r*osmarinifolia* creating numerous, closely arranged and straight, multi-branching stems. Simultaneously, it is worth mentioning that the lower production of generative stalks in the vicinity of strong competitors has been noticed in *Plantago coronopus* [52].

Traits connected with generative propagation

The observed low height of generative stems of *Betonica officinalis* in meadow in the present study presumably allows for diminishing of the risk of fracture by wind in an open habitat, while their greater size in other sites might contribute to an improvement of the pollination and seed dispersal in a shaded and crowded environment. The obtained results support preliminary investigations [31]. Additionally, it is worth mentioning that the also closely related to *Betonica officinalis* taxa from the *Lamiaceae* family might manifest an increase of height of the generative stems as an effect of low-irradiance treatment [53-56]. Furthermore, a similar phenomenon was observed in other meadow taxa, such as *Gentiana pneumonanthe* [57] and *Gladiolus imbricatus* [58].

The lack of temporal and spatial variability of the number of branches do not support the observations reporting the shade-induced reduction of branch number in trees [59], shrubs [60, 61], herbaceous plants [62-65], as well as mosses [66]. The aforementioned authors argued that, at a high irradiance level, the main shoot creates numerous branches, whereas shading triggers a reduction of their number, giving priority to the main axis.

The greater dimensions of inflorescences and length of flowers of *Betonica officinalis* in consecutive sites noticed in the present investigation confirms my previous observations [31]. The augmentation of size of inflorescences and flowers might contribute to an increase of pollinator visits, promoting pollen transfer and affecting the mating success of plants in crowded environments. Moreover, the increase of bumble-bee visits with an augmentation of size of vertically-oriented inflorescences and dimensions of flowers was noticed in populations of other species such as *Corydalis ambigua* [67] and *Delphinium* sp. [68]. The aforementioned authors argued that a bee's eyes have limited revolving power, depending on the insect's body size and the object's colour. Thus, bees probably detect plants with large outlines more readily than smaller plants. Additionally, it should be pointed out that the lower number and dimensions of flowers in Patch V might be due to full shade by adjacent shrub willows and trees.

Cauline and rosette leaves

The shade avoidance syndrome in individual *Betonica officinalis* might be manifested by a greater number of nodes with cauline leaves and greater dimensions of the cauline leaves. The creating of larger cauline leaves as an effect of substantial shading was found in a closely related species from genus *Lamium* [53], as well as in other taxa such as *Campanula alliariifolia* [69] and *Valeriana jatamansi* [30]. Moreover, a similar phenomenon was observed in an aquatic plant *Potamogeton richardsonii* [70].

The observed temporal and spatial increase of the number and dimensions of leaf rosettes of *Betonica officinalis* might also be interpreted as a functional response to the growing level of shading. The obtained findings are in accordance with my previous observations [31]. Moreover, it should be pointed out that the creation of much longer leaves under a shading regime than in the full light was observed in several hemicryptophytes, such as *Taraxacum officinale* [71], *Saxifraga nivalis* and *S. moschata* [72].

Simultaneously, it should be noted that the lower number and dimensions of rosette and cauline leaves of *Betonica officinalis* in meadow might suppress water loss through transpiration. It is a well-known phenomenon adjusting the balance between water availability and intense solar radiation can reduce the number and size of leaves [73, 74].

Conclusions

Summarizing, the majority of traits (the total number of aboveground units, share of leaf rosettes and generative stems, number and dimensions of leaf rosettes, number of cauline leaf nodes, dimensions of cauline leaves, height of generative stems, length of flowers) is constant in the consecutive years, while differ among the study sites. The length and width of the rosette

leaves as well as the smaller length present both temporal and spatial variability, while the number of generative stem branches does not change either in time or in space. Ramet clusters growing in sunny stands are characterised by a considerable number of generative stems, a modest length of inflorescences and flowers, as well as a lower number and size of leaves. Ramet clusters occurring in a competitive neighbourhood show a lower number of aboveground units, a slighter share and more substantial height of generative stems, large inflorescences and flowers, as well as more substantial number and greater dimensions of leaves. Due to morphological plasticity, the established clusters of *Betonica officinalis* might adapt to environmental conditions i.e. they might spread in open stands as well as endure in crowded environments.

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